Evaluation of Inflammatory Related Markers in Gestational Diabetes Mellitus

Inflamasyonla İlişkili Belirteçlerin Gestasyonel Diyabet Mellitus'ta Değerlendirilmesi

Gestasyonel Diyabet Mellitusta Inflamasyon

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Özet

Amaç: Subklinik inflamasyonun, gestasyonel diyabet mellitus (GDM) patofizyolojisinde önemli bir faktör olduğu düşünülmektedir. İnflamatuvar durumun değerlendirilmesinde nötrofil-lenfosit oranı (NLO) ve lenfosit-monosit oranının (LMO) ölçülmesi basit ve ucuz bir metottur. Bu çalışmada GDM'li hastalarda NLO, LMO ve diğer sık kullanılan diğer inflamatuvar belirteçlerin değerlendirilmesi ayrıca NLO ve LMO'nun metabolik parametrelerle korelasyonunun olup olmadığının saptanması hedeflenmiştir. Gereç ve Yöntem: Bu kontrollü kesitsel çalışmada 35 tekil, ardışık GDM tanısı almış gebe ve 40 komplikasyonsuz, sağlıklı, vücut kitle indeksi ve yaş açısından hasta grubuyla eşleştirilmiş kontrol gebesi değerlendirildi. GDM tanısı için oral glukoz tolerans testi (OGTT) 24-28. gebelik haftalarında gerçekleştirildi. Yüksek duyarlılıklı C reaktif protein (hs-CRP), NLO, LMO, açlık kan şekeri (AKŞ), açlık insülini ve insülin rezistansının hemostaz modelle değerlendirilmesi (HOMA-IR) değerleri her 2 grupta karşılaştırıldı. Bulgular: Sağlıklı hastalarla karşılaştırıldığında GDM'li gebelerde NLO daha yüksek iken LMO oranı daha düşüktü. GDM'li gebelerde hs-CRP seviyeleri yüksek bulunsa da bu fark istatistiksel olarak anlamlı değildi (p= 0.09). HbA1c, insülin ve HOMA-IR sağlıklı gebelerle karşılaştırıldığında GDM'li grupta anlamlı olarak yüksek iken AKŞ değerleri her 2 grupta benzerdi. Tartışma: Sistemik inflamasyon GDM ile ilişkilidir ve bu ilişki gebelikteki kilo alımı ve annenin vücut kitle indeksinden bağımsızdır. Bu çalışmadaki sonuçlar GDM patogenezinde inflamasyonun önemli bir rolü olabileceğini düşündürmüştür.

Anahtar Kelimeler

İnflamasyon; İnsülin Rezistansı; Lenfosit; Nötrofil; C Reaktif Protein

Abstract

Aim: Subclinical inflammation has been suggested as an important factor in the pathophysiology of gestational diabetes mellitus (GDM). Measuring the neutrophil-to-lymphocyte ratio (NLR) and lymphocyte-to-monocyte ratio (LMR) may be a simple and inexpensive method for assessing inflammatory status. The aim of this study is to evaluate the NLR and LMR and other commonly used inflammatory markers in women with GDM and to determine the correlation of NLR and LMR with metabolic parameters in gestational diabetic women. Material and Method: In this controlled cross-sectional study we examined 35 singleton, consecutive pregnant women with GDM and a control group of 40 healthy women with uncomplicated pregnancies, matched for body mass index and age. Oral glucose tolerance tests (OGTT) were performed at 24-28 weeks of pregnancy to diagnose GDM. The high-sensitive C-reactive protein (hs-CRP), NLR and LMR, fasting blood glucose (FBG), fasting insulin, and homeostatic model assessment of insulin resistance (HOMA-IR) scores of the two groups were also compared. Results: NLR levels were higher and LMR levels were lower in pregnant with GDM women compared with healthy ones. Although hs-CRP levels were found to be higher in the GDM group, this difference was not statistically significant (p=0.08). While HbA1c, insulin, and HOMA-IR scores were significantly elevated in the GDM group compared with the healthy pregnant group, FBG levels were comparable. Discussion: Systemic inflammation was associated with GDM, and the association was independent of maternal BMI and weight gain during pregnancy. The results of this study suggest that inflammation plays an important role in the pathogenesis of GDM.

Keywords

Inflammation; Insulin Resistance; Lymphocyte; Neutrophil; C-Reactive Protein

DOI: 10.4328/JCAM.4429 Received: 23.02.2016 Accepted: 09.03.2016 Printed: 01.07.2016 J Clin Anal Med 2016;7(4): 501-4
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Introduction

Gestational diabetes mellitus (GDM) is defined as "any degree of glucose intolerance diagnosed in pregnancy." Like Type 2 diabetes, GDM is characterized by the metabolic defects of beta-cell dysfunction and insulin resistance [1]. Cumulating data suggest that women with GDM have an increased risk of maternal and fetal complications during pregnancy as well as longer-term risks including a higher prevalence of type-2 DM and cardiovascular disease in later life [2]. Inflammatory mediators such as C- reactive protein (CRP), IL-6, and TNF-α are associated with type 2 and gestational diabetes. Although currently available data regarding inflammation in women with GDM are still conflicting, it has been hypothesized that adipose tissue leads to immune and inflammatory responses of both white adipose tissue and the placenta, contributing to systemic inflammation [3, 4].

Systemic inflammation can be measured using a variety of hematological markers. Recent findings indicate that blood cell subtype ratios, such as the neutrophil-to-lymphocyte ratio (NLR) and the lymphocyte/ monocyte ratio (LMR) may provide prognostic and diagnostic clues to diseases related to chronic low-grade inflammation [5-7].

The primary aim of this study is to evaluate the potential role of inflammation related parameters including NLR and LMR in GDM. We also investigate the markers' association with metabolic parameters in gestational diabetic women.

Material and Method

This cross-sectional study was conducted at Zekai Tahir Burak Research and Training Hospital between January and November 2015. The Institutional Review Board of the hospital approved the study and the universal principles of the Helsinki Declaration were applied. Informed consent was obtained from all of the participants.

All of the pregnant women were examined for infection, and patients with any signs and symptoms of active infection (pain, fever, or vaginal discharge) were excluded from the study. Also excluded were women with pre-existing conditions (or a history thereof) including chronic hypertension; pre-pregnancy diabetes mellitus or a complication of gestational diabetes mellitus in a previous pregnancy; other cardiovascular, endocrinological, urogenital, gastrointestinal, autoimmune, or oncological diseases; thrombophilia; or multiple gestation. In addition, women who used medications such as glucocorticoids that could interfere with blood glucose, and women with pre-gestational obesity (BMI >30 kg/m2) were excluded.

Gestational age was determined based on the first day of the last menstrual period and first trimester ultrasonographic measurement of the crown–rump length. At our center, all pregnant patients are screened for GDM at 24–28 weeks' gestation using the two-step approach. All patients underwent a standard oral glucose tolerance test (OGTT), with the use of a 50-gram dose of glucose, between 24 and 28 weeks. If the 1-hour value was 140 mg/dl or greater, patients were further evaluated with 100 g OGTT, which was performed in the morning after an overnight fasting. The results were evaluated according to the Carpenter and Coustan criteria (0-h 95 mg/dL, 1-h 180 mg/dL, 2-h 155 mg/dL, 3-h 140 mg/dL) [8]. A 50 g OGTT value of 200 mg/dL or higher was also diagnostic for GDM. The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated using the following formula: FG (mg/dl) × Fasting insulin level (μU/ml) / 405 [9].

All CBC analyses were conducted in the central hematology laboratory of the hospital, using a Gen-S automated analyzer. The NLR value was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. The LMR value was calculated by dividing the absolute lymphocyte count by the absolute monocyte count. Plasma concentrations of high-sensitivity CRP (hs-CRP) were determined with a Tinaquant CRP (Latex) high-sensitive particle-enhanced immuno turbidimetric assay on a Roche Modular P analyzer (Roche kit; Roche Diagnostics) according to manufacturer instructions. Minimum detectable concentration of hs-CRP was 1x10−5 mg/L. All samples were assessed in duplicate.

Pregnant patients who were diagnosed with GDM consulted a dietician for a daily diet programme and an endocrinologist for instructions for the self-monitoring of glucose. Perinatal outcome parameters such as gestational age at delivery, birth weight, preterm delivery, and other perinatal clinical characteristics were also recorded.

The statistical analyses were performed using IBM SPSS Statistics version 15 (IBM Corp., Armonk, NY). Normal distribution of data was assessed using the Kolmogorov–Smirnov test. Continuous and normally distributed variables were presented as mean ± standard deviation (SD) and intragroup differences were investigated using one-way analysis of variance. The data were summarized as mean ± standard deviation and median (minimum–maximum). Continuous variables were examined using Kruskal–Wallis tests if the data were not normally distributed. Categorical variables were expressed as number (percentage). Proportions were compared with Fisher's exact test or the chi-square test where appropriate. Pearson's correlation analysis was used to study the correlations between measurements. Diagnostic performance (i.e., sensitivity, specificity, and positive and negative predictive values) at the best cutoff point for NLR and LMR was calculated. A two-sided p value <0.05 was considered statistically significant.

Results

Maternal demographic and clinical characteristics are shown in Table 1. Age, gravidity, pre-pregnancy BMI, and weight gain during pregnancy in the two groups of patients were similar (p>0.05 in all pairwise comparisons).

Although there were no statistically significant differences between the groups in HbA1c and FBG, the fasting insulin levels and HOMA-IR values were significantly higher in the GDM group than in the control group (p= 0.02 and p<0.001, respectively).

While the mean WBC and hs-CRP values were comparable between the groups, the mean NLR levels were significantly higher and LMR levels were significantly lower in the GDM group compared with the control group (Table 2).

The comparison of the perinatal outcomes of the GDM and control groups is depicted in Table 3.

At a cutoff level of 4.34, NLR accurately diagnosed GDM [AUC=0.568 (95% confidence interval 0.224–0.912), p=0.005] with sensitivity and specificity rates of 65% and 58% and posi-

tive and negative predictive values of 63.6% and 62.3%, respectively.

Table 1. Characteristics of the groups.

	Control Group (n=40)	GDM (n=35)	p value
Age, years	27.9 (20-34)	26.8 (20-34)	NS
Gravidity, range	2 (0-3)	2(1-3)	NS
Parity, range	1 (0-2)	2(1-3)	NS
Any Prenatal Smoking, n (%)	2(5%)	2(5.6%)	NS
Pre-pregnancy BMI, kg/m2	22.1± 3.7	23.5± 4.9	NS
Maternal weight gain during pregnancy, kg	12± 4.9	14.1± 7.5	0.08
BMI at assessment, kg/m2	24.6± 3.8	25.3± 4.4	NS
Gestational week at assessment, range	26.3 (24-28)	25.7 (24.4-28)	NS
50- Gram OGTT, mg/dl	117± 19	168± 27	<0.01
100- Gram OGTT, mg/dl			
Fasting glucose, mg/dl	78.7± 6.3	82.8± 9.7	NS
1 hour	-	181± 19	-
2 hour	-	178± 31	-
3 hour	-	145± 28	-

Data expressed as mean ± SD; the mean difference is significant at the 0.05 level. NS: Non-significant.
BMI; Body Mass Index.

Table 2. Biochemical, hematological and Inflammation related markers in the groups.

	Control Group (n=40)	GDM (n=35)	p value
Hemoglobin (g/dL)	12.9± 1.8	12± 3.6	NS
Hs-CRP (mg/ L)	4.9± 3.5	5.70± 2.1	0.09
WBC count (x103/mm3)	9.9± 2.6	10.2± 3.1	NS
NLR	3.97± 1.34	4.54± 2.98	0.04
LMR	3.65± 1.22	3.26± 1.21	0.05
HbA1c (%)	4,56± 0,26	5,38± 0,33	0.04
Fasting blood glucose (mg/dl)	78.7± 6.3	82.8± 9.7	NS
Fasting insulin (µU/ml)	7.2± 2.1	10.9± 2.9	<0.001
HOMA-IR	2.53± 0.33	3.66± 0.21	0.02

Data expressed as mean ± SD; the mean difference is significant at the 0.05 level. NS: Non-significant.
CRP; C-reactive protein, HbA1c; glycated hemoglobin, HOMA-IR; homeostatic model assessment of insulin resistance, LMR; lymphocyte to monocyte ratio, NLR; Neutrophil/lymphocyte ratio, WBC; white blood cell.

Table 3. The comparison of the perinatal outcomes in the control and GDM cases

	Control Group (n= 40)	GDM (n= 35)	p value
Gestational week at delivery	40.2 (36-41)	38.4 (36-39)	0.02
Birth weight (grams)	3582± 650	3497± 844	NS
Mode of Delivery			
Vaginal	25 (62.5%)	11 (31.4%)	<0.001
C/S	15 (37.5%)	24 (68.6%)	<0.001
Preterm Delivery, n(%)	2 (5%)	6 (17 %)	<0.001
Birth weight >90th percentile	-	4 (11.4 %)	<0.001
5 minute Apgar ≤ 7	-	5 (14 %)	<0.001
NICU admission	1 (2.5 %)	5 (14 %)	<0.001

Data expressed as mean ± SD; the mean difference is significant at the 0.05 level. NS: Non-significant.
C/S; Cesarean section, NICU; Neonatal intensive care unit.

Discussion

To the best of our knowledge, this is the first study to explore the possible relationships among LMR, NLR, and other routine hematologic parameters and CRP, FI, FBG, HOMA-IR and GDM. The findings of this study are as follows: 1) NLR levels were higher and LMR levels were lower in pregnant with GDM women compared to healthy ones; 2) Although hs-CRP levels were found to be higher in the GDM group, this difference is not statistically significant (p=0.09); 3) While HbA1c, insulin and HOMA-IR levels were significantly increased in the GDM group compared with the healthy group, FBG levels were comparable between the groups.

Previous studies have shown that GDM can be regarded primarily as a condition of insulin resistance [10]. Furthermore, it has been reported that women with GDM have an increased risk of developing type 2 DM compared with those who have a normoglycemic pregnancy (RR 7.43, 95% CI 4.79-11.51) [11]. Consistent with the literature, in this study we show increased levels of HbA1c, insulin, and HOMA-IR in those pregnant with GDM compared to those without GDM.

In recent years, the role of immune activation and inflammation in the pathogenesis of GDM has gained increasing attention [12]. Pregnancy itself is associated with a changed inflammatory profile compared to the non-pregnant state. A tightly regulated balance between pro- and anti-inflammatory statuses may be necessary for normal implantation, invasion of the extravillous trophoblast, and placentation [13]. Exaggerated inflammation has been blamed in the pathogenesis of pregnancy complications such as pre-eclampsia and GDM [4]. Taken together, these data support a possible role for inflammation in the pathogenesis, although direct evidence of causality is lacking.

Hs-CRP, one of the most commonly measured markers of inflammation for the evaluation and treatment of inflammatory disorders, is also associated with GDM and Type 2 diabetes [14,15]. It has been shown that lean women with CRP > or = 5.3 mg/L experience a 3.7-fold increased risk of GDM [95% CI 1.6, 8.7] as compared with women with CRP < 5.3 mg/L [5]. In the current study, although hs-CRP values were higher in the GDM than in the control group (4.9± 3.5 vs 5.70± 2.1 mg/ L), this difference is not significant (p=0.09). The limited number of patients in this study might be the reason we did not find significant differences in maternal serum hs-CRP levels. The role for HbA1c analysis during pregnancy is not yet established and the use of HbA1c measurement in GDM is controversial. HbA1c measurement is typically used to monitor control in pregnant women with pre-pregnancy diabetes [16,17]. In our study, we found significantly higher levels of HbA1c in women who were diagnosed with GDM according to the Carpenter and Coustan criteria [8].

Individual leukocyte subpopulations represent single elements of the leukocyte differential and immunological processes. It is well known that neutrophils, lymphocytes, and monocytes play important roles in inflammation. NLR has been evaluated recently as an inflammatory marker in several diseases and as a marker for several types of cancer [5-7,18]. It has been shown that women who developed GDM had significantly higher neutrophil, lymphocyte, platelet, and erythrocyte counts before 20

weeks gestation [19]. However, the LMR and its possible value as a prognostic marker have not been evaluated in GDM before. Relative lymphopenia reflects a physiologic stress response, whereas monocytosis reflects a chronic systemic inflammation. It has been found recently that a low LMR is associated with increased mortality in heart failure [20] and several types of cancer [21]. In this study, for the first time in the literature, we demonstrate significantly lower levels of LMR in patients with GDM, suggesting that inflammation has an important role in GDM.

There are some limitations in our study that should be acknowledged. First, it was a single-center study with a small sample size. Therefore, it is possible that the findings may not be completely generalizable to a broader population. The strengths of our study are the homogeneity of the characteristics of the women in the study groups and its prospective design. All of the pregnant women were examined for infection and body temperature was measured before obtaining blood samples. Patients with any signs and symptoms of active infection (pain, fever, or vaginal discharge) were excluded from the study. Moreover, in the present study, since the BMI and the maternal age were comparable between the groups, the possible confounding effects were eliminated. Nevertheless, considering that laboratory work-up is very simple and inexpensive and that results are available soon after admission, measuring NLR and LMR may provide important prognostic information.

Competing interests

The authors declare that they have no competing interests.

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How to cite this article:
Kirbas A, Daglar K, Danisman N. Evaluation of Inflammatory Related Markers in Gestational Diabetes Mellitus. J Clin Anal Med 2016;7(4): 501-4.